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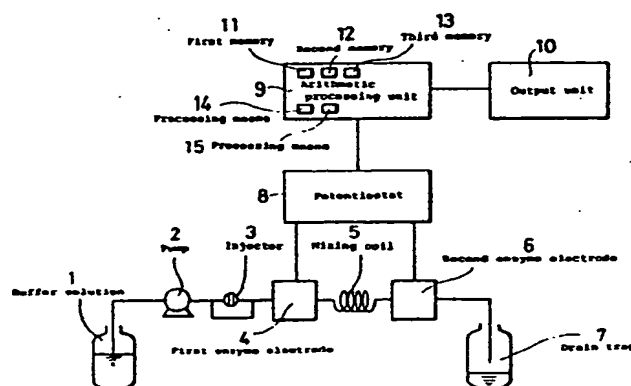
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Measuring apparatus of two components using enzyme electrodes and the measuring method thereof.

In a two-component measuring apparatus employing a first enzyme electrode (4) responding only to a first component to be measured and a second enzyme electrode (6) responding to both first and second components to be measured, in which the enzymatic reactions for forming detectable substances are common and the substances to be finally detected by the electrodes (4, 6) are identical, when calculating the calibration curve for the second component to be measured, the output portion due to traces of the first component contained in the second component to be measured is removed, so that the second component is measured at high sensitivity and high accuracy.

Fig.2



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MEASURING APPARATUS OF TWO COMPONENTS USING ENZYME ELECTRODES AND THE MEASURING METHOD THEREOF.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a measuring apparatus using enzyme electrodes for simultaneously measuring the concentration of two components such as glucose and sucrose in a liquid and its method, and more particularly to the improvement of measuring apparatus of two components and the method thereof wherein the enzymatic reaction for forming detectable substance in each electrode is common, and the substance to be finally detected by each electrode is identical.

2. Description of the Prior Art

Enzyme electrodes are excellent in readiness and promptness; and have come to be used widely in various fields including clinical analysis, food analysis and environmental analysis.

Recently, in particular, great efforts are concentrated in the development of measuring apparatus capable of measuring the concentration of two different substances using enzyme electrodes, and simultaneous measuring apparatus for measuring, for example, glucose and uric acid (the Japanese Laid-open Patent No. 62-24142), lactic acid and pyruvic acid (the Japanese Laid-open Patent No. 62-5172), and others have been introduced, in which each of the employed enzymes function as the catalyst for reaction with a specific object of measurement.

And also, apparatus for measuring the concentration of two components simultaneously was disclosed by the present inventors, wherein the enzymatic reaction for forming or consuming detectable substance in each electrode is common, and the substance to be finally detected by each electrode is identical (the Japanese Laid-open Patent No. 64-69944, EP0310824).

Measuring method of sucrose is disclosed, in which the sucrose is hydrolyzed by invertase into fructose and α -D-glucose, and

α -D-glucose is converted into β -D-glucose by mutarotase, and then β -D-glucose is oxidized by glucose oxidase to produce an electrode active substance of hydrogen peroxide, which is detected electrochemically (C. Bertrand, P. R. Coulet, D. C. Gautheron, Anal. Chim. Acta, 126, 23-34, 1981). On the other hand, to measure the concentration of glucose, the glucose is oxidized by glucose oxidase, and the produced hydrogen peroxide is electrochemically detected. That is, both in the measurement of sucrose and the measurement of glucose, the oxidation of glucose is a common reaction to produce an electrode active substance, and the substance detected by the electrode is hydrogen peroxide.

Therefore, present inventors disclosed the method to measure simultaneously glucose and sucrose by using enzyme electrodes. The concentration of glucose is determined from the output voltage detected by the enzyme electrode having immobilized glucose oxidase. To measure the sucrose concentration, on the other hand, the enzyme electrode for detecting both glucose and sucrose having immobilized glucose oxidase, mutarotase and invertase is set in a flow type measuring apparatus. And by using the previously detected concentration of glucose, the contribution portion of the glucose initially contained in the sample is calculated from the preliminarily calibrated value, concerning the enzyme electrode having immobilized glucose oxidase, mutarotase and invertase, and this value is subtracted from the output current value to determine the sucrose concentration.

That is, generally, the concentrations of two components are measured in the following methods.

Calibration means 1

First of all, using standard solutions of a first component to be measured at several concentrations, output currents are measured by a first enzyme electrode and a second enzyme electrode.

In the specification herein, the first component to be measured refers to a substance which forms by enzymatic reaction a detectable substance by the electrode. The second component to be measured, which is mentioned later, refers to a substance which does not produce an electrode detectable substance directly

by the above mentioned enzymatic reaction, but is capable of producing the first component to be measured by different enzymatic reactions.

Furthermore, the first enzyme electrode is an electrode having immobilized an enzyme catalyzing the reaction to produce an electrode detectable substance from the first component to be measured. And the second enzyme electrode is an electrode having an immobilized enzyme catalyzing the reaction to produce the first component to be measured from the second component to be measured, and an immobilized enzyme catalyzing the reaction to produce an electrode detectable substance from the first component to be measured.

In amperometry, the concentration and output current are in proportional relation. And from the concentrations and the output current in the first enzyme electrode, a formula (1) (used as a first calibration curve for determination of the first component to be measured, as shown in Fig. 1 (1)) is obtained.

$$d1 = B1 \times c1 + A1 \quad (1)$$

where c1: concentration of the first component to be measured

d1: output current of the first enzyme electrode corresponding to the first component to be measured

A1, B1: constants of calibration curve corresponding to the first component to be measured of the first enzyme electrode

Likewise, from the concentrations and output current in the second enzyme electrode, a formula (2) (used as a second calibration curve for detecting the output current in the second enzyme electrode of the first component to be measured, as shown in Fig. 1 (2)) is obtained.

$$d2 = B2 \times c1 + A2 \quad (2)$$

where c1: concentration of the first component to be measured

d2: output current by the first component to be measured of second enzyme electrode

A2, B2: constants of calibration curve corresponding to the first component to be measured of the second enzyme electrode

Next, by measuring the standard solution of the second component to be measured at several concentrations by the second enzyme electrode, from the concentrations and the output current in the second enzyme electrode, a formula (3) (used as a third calibration curve for determination of the second component to be measured, as shown in Fig. 1 (3)) is obtained.

$$d3 = B3 \times c2 + A3 \quad (3)$$

where c2: concentration of the second component to be measured

d3: output current by the second component to be measured of the second enzyme electrode

A3, B3: constants of calibration curve corresponding to the second component to be measured of the second enzyme electrode

35

Determination means 1

Measuring a sample solution containing first component to be measured and second component to be measured, both of unknown concentration, by two enzyme electrodes, the output current d1 attributable to the first component to be measured is obtained in the first enzyme electrode, and the output current d23 attributable to both first component to be measured and second component to be measured is obtained in the second enzyme electrode.

The concentration c1 of the first component to be measured is obtained by putting the output current d1 in the first enzyme into formula (1), as shown in formula (a).

$$c1 = (d1 - A1) / B1 \quad (a)$$

(See Fig. 1 (1).)

Next, the concentration of the second component to be measured is obtained in the following sequence. The output current of the second enzyme electrode which is attributable to the first component is calculated from the formula (2) and is obtained as correction value d2 as shown in formula (b) (see Fig. 1 (2)), and this correction value d2 is subtracted from the output current d23 obtained in the second enzyme electrode to obtain d3 (the current purely attributable to the second component to be measured) as shown in formula (c), and this value is put into formula (3) to obtain the desired concentration as shown in formula (d) (see Fig. 1 (3)).

$$d2 = \{B2 \times (d1 - A1) / B1\} + A2 \quad (b)$$

$$d3 = d23 - d2 \quad (c)$$

$$c2 = (d3 - A3) / B3 \quad (d)$$

The standard solution to be used in plotting of calibration curve is required to be a solution of a purely single component with a known concentration, but when measuring, for example, glucose and sucrose

simultaneously as mentioned above, glucose is often contained as impurity in the standard solution of sucrose. And in the second enzyme electrode a small current derived from glucose contained in the sucrose standard solution is obtained, and therefore sucrose, which is second component to be measured, cannot be measured accurately.

Actually in a system where the substances to be finally detected by the electrodes are identical, the first component to be measured is often contained as impurity in the standard solution of the second component to be measured, and it is extremely difficult to remove this impurity or detect the content in advance, and since the substance used as the standard solution gradually produces impurities little by little as the time passes (for example, sucrose produces glucose), it is practically impossible to remove or detect the impurities preliminarily. Therefore, in the measuring method of the prior art, the correct concentration of the second component to be measured cannot be obtained. This problem becomes more serious when the sensitivity of the measuring apparatus is higher.

Presented hereabove is measurement of coexistent system of sucrose and glucose, and similar problems exist in simultaneous measuring methods of two components using enzyme electrodes producing same electrode active substances by the finally common enzymatic reactions, for example measurement of maltose-glucose coexistent system, esterified and free cholesterol coexistent system, and the like.

SUMMARY OF THE INVENTION

It is hence a primary object of the invention to present a measuring apparatus and measuring method for determining the two components at high accuracy by solving the above-discussed problems when measuring two components common in the final enzymatic reaction for producing or consuming electrode active substances.

To achieve the above object, the two-component measuring apparatus of the invention comprises a first enzyme electrode responding only to a first component to be measured, and a second enzyme electrode responding to both first component to be measured and second component to be measured, both enzyme electrodes having enzyme common in the enzymatic reaction for forming a detectable substance at electrodes, identical in the substance to be finally detected by electrodes, and further comprising means for calculating a calibration curve of the second component to be measured, by (i) calculating a contribution portion of the first component to be measured, which is an impurity in the standard solution containing known amount of the second component, in the second enzyme electrode by using the relation of the output values of standard solution containing known amount of the first component in both enzyme electrodes, and (ii) subtracting said contribution portion from the output of the standard solution containing known amount of the second component in the second enzyme electrode.

The invention also presents a two-component measuring apparatus which comprises:

- a. a first enzyme electrode responding only to a first component to be measured;
- b. a second enzyme electrode responding to both first component to be measured and second component to be measured;

- c. a first memory for storing a first calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the first enzyme electrode;

- d. a second memory for storing a second calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the second enzyme electrode;

- e. a third memory for storing a third calibration curve for expressing the relation between the concentration of the second component to be measured and the output level of the second enzyme electrode calculated by f;

- f. processing means for calculating the third calibration curve, said processing means calculates the third calibration curve on the basis of the output levels in the second enzyme electrode of the second component obtained by changing the concentration of the second component to be measured in the standard solutions, and repeats the process in the sequence of f1 to f4;

- f1. detecting the output levels attributable to the standard solution in the first enzyme electrode and the second enzyme electrode, and said standard solution contains the first component to be measured and the second component to be measured with a known concentration of the second component to be measured;

- f2. calculating the concentration of the first component to be measured on the basis of the detected output level in the first enzyme electrode and the first calibration curve stored in the first memory;

- f3. calculating the output level in the second enzyme electrode of the first component to be measured on the basis of the calculated concentration of the first component to be measured and the

second calibration curve stored in the second memory:

f4. calculating the output level in the second enzyme electrode of the second component to be measured by subtracting the calculated output level in the second enzyme electrode of the first component to be measured from the output level in the second enzyme electrode of the standard solution; and

5 g. processing means for calculating the concentrations of the first component to be measured and the second component to be measured of the sample by using the first, second and third calibration curves.

According to the invention, the second enzyme electrode is disposed at the downstream side of the first enzyme electrode, and

a mixing coil is disposed between the first enzyme electrode and the second enzyme electrode in order to
10 diffuse and dilute the first and second components to be measured in the forward and backward directions of the flow.

The invention also relates to a two-component measuring method comprising:

a. a first step of preparing

a1. a first enzyme electrode responding only to a first component to be measured, and

15 a2. a second enzyme electrode responding both to the first component to be measured and to the second component to be measured,

b. a second step of calculating a first calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the first enzyme electrode,

c. a third step of calculating a second calibration curve for expressing the relation between the
20 concentration of the first component to be measured and the output level of the second enzyme electrode,

d. a fourth step of preparing a standard solution containing both the first component to be measured and the second component to be measured, with a known concentration of the second component to be measured,

e. a fifth step of determining the concentration of the first component to be measured contained in
25 the standard solution by using the output level of the first enzyme electrode to the standard solution and the first calibration curve,

f. a sixth step of determining the output level in the second enzyme electrode of the first component to be measured contained in the standard solution by using the concentration of the first component to be measured and the second calibration curve,

30 g. a seventh step of determining the output level in the second enzyme electrode of the second component to be measured, by subtracting the output level in the second enzyme electrode of the first component to be measured from the output level of the second enzyme electrode,

h. an eighth step of calculating a third calibration curve for expressing the relation between the concentration of the second component to be measured and the output level of the second enzyme
35 electrode, by varying the concentration of the second component to be measured in the standard solution, and repeating the procedure in the sequence of fourth step to seventh step, and

i. a ninth step of determining the concentrations of the first and second components to be measured on the basis of the first, second and third calibration curves.

In the invention, the first component to be measured delivers outputs to both first and second
40 electrodes. Indicating the output in the first electrode of the first component to be measured with concentration c to be p_1 and the output in the second electrode to be p_2 , the relation

$$c = f(p_1) \quad (A)$$

$$p_2 = g(p_1) \quad (B)$$

is determined by measuring the standard solution of the first component to be measured, wherein $f()$ and
45 $g()$ denote that c and p_2 are expressed in the function of p_1 . If the second component to be measured is pure, no output is given to the first electrode, but if the first component to be measured is contained as impurity, a current of p_1 is delivered from this first component to be measured to the first electrode. Putting this p_1 into formula (B), the output p_2' of the second electrode due to the impurity will be calculated. Therefore, when p_2' is subtracted from the output of the second electrode of the standard
50 solution of the second component to be measured, the second electrode output purely attributable to the second substance to be measured is obtained, so that, unlike the prior art, a very accurate calibration curve of the second component to be measured is calculated. In the conventional method, the output containing the error of p_2' attributed to the second component in the standard solution was directly taken as the output of the second component to be measured in the second electrode, and the concentration of the second
55 component to be measured was determined by the calibration curve containing the above error and the value obtained by subtracting the output attributed to the first component to be measured, said output obtained by formula (B), from the output of the second electrode, in a measurement of sample containing first and second component to be measured. And hence the concentration was smaller than actual value.

By contrast, in the invention, since the portion of the output due to the impurity is eliminated in the calculation of the calibration curve for the second component to be measured as stated above, a correct concentration of the second component to be measured is obtained.

In other words, according to the invention, in the two-component measuring apparatus comprising the first enzyme electrode responding only to the first component to be measured and the second enzyme electrode responding to both the first component to be measured and the second component to be measured, in said electrodes both contain enzyme and the enzymatic reactions for forming electrode detectable substances are identical, and the substances to be finally detected by the electrodes are identical, any determination error due to the first component to be measured which is a impurity in the second component to be measured does not occur, and therefore measurement of high sensitivity and high accuracy is realized.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram showing a calibration curve used in a conventional two-component measuring apparatus, which is used for explaining the conventional means for determining;

Fig. 2 is a system diagram showing an example of a two-component measuring apparatus of the invention;

Fig. 3 is a diagram showing a calibration curve used in a two-component measuring apparatus of the invention, which is used for explaining the method of plotting a third calibration curve; and

Fig. 4 is a flow chart showing an arithmetic processing unit of the system diagram shown in Fig. 2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Preferred embodiments of the invention are described in details with reference to the drawings attached hereto.

Fig. 2 is a system diagram showing an example of a two-component measuring apparatus of the invention. This embodiment shows a flow type, in which a buffer solution 1 is supplied through a pump 2, the substance to be measured is injected from an injector 3, and it is sent out into a drain trap 7 from a first enzyme electrode 4 through a mixing coil 5 and a second enzyme electrode 6.

To each electrode, a voltage is applied by a potentiostat 8, and a current output based on each component to be measured is obtained, and this information is sent to an arithmetic processing unit 9 by means for generally converting trace current into a digital quantity such as current amplification and by digitizing the output current with an A/D converter, and the operation for determination of calibration curves and concentration is executed, and the result is delivered in a displaying or printing output unit 10. In order to explain, the first memory, the second memory and the third memory were described separately, but they can be memorized at specific address of the same Random Access memory.

By diluting the sample, for example, by intervening a mixing coil 5 between the first enzyme electrode and the second enzyme electrode, the proportional range of the second enzyme electrode is expanded, and the two substances may be measured more accurately even if the concentration is very high (refer to the Japanese Laid-open Patent No. 64-69944, EP0310824).

As detecting methods in measurement, in addition to the amperometric method above mentioned, potentiometric method and thermal measurement method (to measure the changes in enthalpy due to enzymatic reaction) may be possible, and considering from the dynamic range and response speed of these methods, the amperometric method is most preferable.

In the invention, the calibration means in the arithmetic processing unit 9 may be calibrated in the procedure shown in, for example, Calibration means 2 below, and thereafter the sample of unknown concentration is measured in the procedure shown in Determination means 2 below.

Calibration means 2

First of all, output levels of a standard solution of the first component to be measured at several concentrations are measured by the first enzyme electrode and second enzyme electrode.

From the concentrations and the output levels in the first enzyme electrode, formula (4) (used as a first calibration curve for determination of the first component to be measured, as shown in Fig. 3 (1)) is obtained.

$$d1 = B1 \times c1 + A1 \quad (4)$$

where c1: concentration of the first component to be measured

d1: output level of the first enzyme electrode to the first component to be measured

A1, B1: constants of calibration curve of the first enzyme electrode to the first component to be measured

5 Likewise, from the concentrations and output levels in the second enzyme electrode, formula (5) (used as a second calibration curve for detecting the output level in the second enzyme electrode of the first component to be measured, as shown in Fig. 3 (2)) is obtained.

$$d2 = B2 \times c1 + A2 \quad (5)$$

where c1: concentration of the first component to be measured

10 d2: output level of the second enzyme electrode attributed to the first component to be measured

A2, B2: constants of calibration curve of the second enzyme electrode attributed to the first component to be measured

Next, using a standard solution of the second component to be measured at known concentration (c2), the output level d1 attributable to the first component to be measured contained as impurity is measured in the first enzyme electrode, and the output level d23 attributable to both the second component to be measured and the first component to be measured contained as impurity is measured in the second enzyme electrode.

The contributing portion d2' of the first component to be measured in the second enzyme electrode is obtained from formula (4) and (5) (see Fig. 3 (1), (2)),

$$20 \quad d2' = \{B2 \times (d1 - A1) / B1\} + A2$$

and the contributing portion d2' is subtracted from the output current d23 in the second enzyme electrode, thereby calculating d3' as follows:

$$d3' = d23 - d2'$$

Similarly measuring standard solutions of the second component to be measured at several concentrations, formula (6) about the corrected output current and concentration (used as a third calibration curve for determination of second component to be measured, as shown in Fig. 3 (3)) is obtained.

$$25 \quad d3' = B3' \times c2 + A3' \quad (6)$$

where c2: concentration of the second component to be measured

d3': true output level of the second enzyme electrode attributable to the second component to be measured

30 A3', B3': constants of calibration curve of the second enzyme electrode to the second component to be measured

Thus, the first, second and third calibration curves are plotted automatically, and are stored in first, second and third memories in the arithmetic processing unit. The calibration curves may be used in a certain small error range as far as the experimental conditions are not changed.

35 However, the enzymatic reaction changes its rate when the pH of the buffer solution is changed only slightly even if the temperature, sample volume and flow rate are constant. In actual measurement, considering conditions of the parts of the apparatus, for example, temperature fluctuations in the detecting unit due to ambient temperature or slight variation of the pump flow rate, it is desired to calculate the calibration curve just before measurement of the sample from the viewpoint of measuring at high precision.

40 Incidentally, although different due to the capacity of the memory and purpose of use of the apparatus, it may be also possible to store the calibration curves for plural sets of objects of measurement, and select and use proper calibration curves when the electrodes are exchanged. In this method, however, due caution is needed because the number of condition fluctuation factors may increase.

45

Determination means 2

A sample solution containing the first component to be measured and the second component to be measured, both of unknown concentrations, is measured by two enzyme electrodes, and the output level d1 attributable to the first component to be measured is obtained in the first enzyme electrode, and the output level d23 attributable to both the first component to be measured and the second component to be measured is obtained in the second enzyme electrode.

The concentration of the first substance to be measured c1 is obtained by putting the output level d1 in the first enzyme electrode into formula (4) as shown in formula (a).

$$55 \quad c1 = (d1 - A1) / B1 \quad (a)$$

Next, the concentration of the second component to be measured is obtained by first calculating the output level expected for the first component to be measured in the second enzyme electrode from the formula (5) as shown in formula (b) to be obtained as correction value d2. Then subtracting this correction

value d2 from the output level d23 obtained in the second enzyme electrode as shown in formula (c) to obtain d3 (the level purely attributable to the second substance to be measured), and putting this value in formula (6) as shown in formula (d).

$$d2 = \{B2 \times (d1 - A1) / B1\} + A2 \quad (b)$$

$$d3 = d23 - d2 \quad (c)$$

$$c2 = (d3 - A3) / B3 \quad (d)$$

The invention of two-component measuring apparatus was conducted, by the amperometric measurement according to the Calibration means 2, using distilled water as control sample and also using glucose standard solutions of 10, 20, 300 mmol/l, and sucrose standard solutions of 10, 20, 30 mmol/l. And said apparatus was installed with the first enzyme electrode having immobilized glucose oxidase, and the second enzyme electrode having immobilized invertase, mutarotase and glucose oxidase. Afterwards, instead of using the solutions of unknown concentrations, using again the glucose standard solutions of 10, 20, 30 mmol/l and sucrose standard solutions of 10, 20, 30 mmol/l, each measurement was repeated three times according to the Determination means 2, and the results are shown in Table 1.

Besides, by way of comparison, the results of measurement according to the Calibration means 1 and Determination means 1 which lacks correction of the calibration curve of the second component to be measured are shown in Table 2.

As known from Table 1 and 2, in the measurement employing the two-component measuring apparatus of the invention, the error of the determination of the second substance to be measured has been notably improved.

Table 1

Substance measured	Results of measurement					
	(Unit: mmol/l)					
	Glucose			Sucrose		
	1st	2nd	3rd	1st	2nd	3rd
10 mmol/l glucose	10.12	9.85	9.8	0.02	0.35	0.40
20 mmol/l glucose	20.15	19.97	19.89	0.00	0.11	0.37
30 mmol/l glucose	30.16	29.94	29.73	0.00	0.00	0.00
10 mmol/l sucrose	0.39	0.37	0.33	10.18	9.99	9.88
20 mmol/l sucrose	0.78	0.79	0.75	20.08	19.91	19.59
30 mmol/l sucrose	0.65	0.68	0.68	29.75	30.34	30.00

Table 2

Substance measured	Results of measurement					
	(Unit: mmol/l)					
	Glucose			Sucrose		
	1st	2nd	3rd	1st	2nd	3rd
10 mmol/l glucose	10.12	9.85	9.81	0.00	0.15	0.19
20 mmol/l glucose	20.15	19.97	19.89	0.00	0.00	0.16
30 mmol/l glucose	30.16	29.94	29.73	0.00	0.00	0.00
10 mmol/l sucrose	0.39	0.37	0.33	9.65	9.46	9.36
20 mmol/l sucrose	0.78	0.79	0.75	19.21	19.05	20.01
30 mmol/l sucrose	0.65	0.68	0.68	29.46	29.14	28.81

The invention has been hitherto mainly illustrated in the measurement of sucrose and glucose coexistent system, but the invention also brings about similar effects by using appropriate enzyme electrodes even in simultaneous measurement of two components using enzyme electrodes producing same electrode active substance by the finally common enzymatic reactions in maltose and glucose coexistent system, esterified and free cholesterol coexistent system, and others.

That is, if the first component is glucose and the second component is sucrose, the first enzyme electrode is an electrode having immobilized glucose oxidase, and the second enzyme electrode is an electrode having immobilized glucose oxidase, mutarotase and invertase, as stated above.

Besides, if the first component is glucose and the second component is maltose, the first enzyme electrode is an electrode having immobilized glucose oxidase, and the second enzyme electrode is an electrode having immobilized glucose oxidase, mutarotase and α -glucosidase.

If the first component is glucose and the second component is β -glucoside, the first enzyme electrode is an electrode having immobilized glucose oxidase, and the second enzyme electrode is an electrode having immobilized glucose oxidase and β -glucosidase.

If the first component is glucose and the second component is maltooligosugars, the first enzyme electrode is an electrode having immobilized glucose oxidase, and the second enzyme electrode is an electrode having immobilized glucose oxidase and glucoamylase.

If the first component is glucose and the second component is lactose, the first enzyme electrode is an electrode having immobilized glucose oxidase, and the second enzyme electrode is an electrode having immobilized glucose oxidase and lactase.

For detection of free cholesterol and esterified cholesterol, the first enzyme electrode is an electrode having immobilized cholesterol oxidase, and the second enzyme electrode is an electrode having immobilized cholesterol oxidase and cholesterol esterase.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description and all changes which come within the meaning and the range of equivalency of the claims are therefore intended to be embraced therein.

Claims

1. A two-component measuring apparatus comprising: a first enzyme electrode (4) responding only to a first component to be measured, and a second enzyme electrode (6) responding to both first component to be measured and second component to be measured, both enzyme electrodes (4, 6) having enzyme common in the enzymatic reaction for forming a detectable substance at the electrodes (4,6) identical in the substance to be finally detected by the electrodes, (4,6) and further comprising means (9) for calculating a calibration curve of the second component to be measured, by (i) calculating a contribution portion of the first component to be measured, which is an impurity in the standard solution containing known amount of the second component, in the second enzyme electrode (6) by using the relation of the output values of standard solution containing known amount of the first component in both enzyme electrodes, (4,6) and (ii) subtracting said contribution portion from the output of the standard solution containing known amount of the second component in the second enzyme electrode (6).

2. A two-component measuring apparatus comprising:

- a. a first enzyme electrode (4) responding only to a first component to be measured;
- b. a second enzyme electrode (6) responding to both first component to be measured and second component to be measured;
- c. a first memory (11) for storing a first calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the first enzyme electrode (4);
- d. a second memory (12) for storing a second calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the second enzyme electrode (6);
- e. a third memory (13) storing a third calibration curve for expressing the relation between the concentration of the second component to be measured and the output level of the second enzyme electrode (6) calculated by f;
- f. processing means (14) for calculating the third calibration curve, said processing means (14) calculates the third calibration curve on the basis of the output levels in the second enzyme electrode (6) of the second component obtained by changing the concentration of the second component to be measured in

the standard solutions, and repeats the process in the sequence of f1 to f4;

f1. detecting the output levels attributable to the standard solution in the first enzyme electrode (4) and the second enzyme electrode, (6) and said standard solution contains the first component to be measured and the second component to be measured with a known concentration of the second component to be measured;

f2. calculating the concentration of the first component to be measured on the basis of the detected output level in the first enzyme electrode (4) and the first calibration curve stored in the first memory (11);

f3. calculating the output level in the second enzyme electrode (6) of the first component to be measured on the basis of the calculated concentration of the first component to be measured and the second calibration curve stored in the second memory (12);

f4. calculating the output level in the second enzyme electrode (6) of the second component to be measured by subtracting the calculated output level in the second enzyme electrode (6) of the first component to be measured from the output level in the second enzyme electrode (6) of the standard solution; and

g. processing means (15) for calculating the concentrations of the first component to be measured and the second component to be measured of the sample by using the first, second and third calibration curves.

3. A two-component measuring apparatus according to claim 1 or 2, wherein the second enzyme electrode (6) is disposed at the downstream side of the first enzyme electrode, (4) and a mixing coil (5) is disposed between the first enzyme electrode (4) and the second enzyme electrode (6) in order to diffuse and dilute the first and second components to be measured in the forward and backward directions of the flow.

4. A two-component measuring method comprising:

a. a first step of preparing

a1. a first enzyme electrode responding only to a first component to be measured, and

a2. a second enzyme electrode (6) responding both to the first component to be measured and to the second component to be measured,

b. a second step of calculating a first calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the first enzyme electrode (4),

c. a third step of calculating a second calibration curve for expressing the relation between the concentration of the first component to be measured and the output level of the second enzyme electrode (6),

d. a fourth step of preparing a standard solution containing both the first component to be measured and the second component to be measured, with a known concentration of the second component to be measured,

e. a fifth step of determining the concentration of the first component to be measured contained in the standard solution by using the output level of the first enzyme electrode (4) to the standard solution and the first calibration curve,

f. a sixth step of determining the output level in the second enzyme electrode (6) of the first component to be measured contained in the standard solution by using the concentration of the first component to be measured and the second calibration curve,

g. a seventh step of determining the output level in the second enzyme electrode (6) of the second component to be measured, by subtracting the output level in the second enzyme electrode (6) of the first component to be measured from the output level of the second enzyme electrode (6),

h. an eighth step of calculating a third calibration curve for expressing the relation between the concentration of the second component to be measured and the output level of the second enzyme electrode, (6) by varying the concentration of the second component to be measured in the standard solution, and repeating the procedure in the sequence of fourth step to seventh step, and

i. a ninth step of determining the concentrations of the first and second components to be measured on the basis of the first, second and third calibration curves.

Fig.1 (1) Prior Art (Determination means 1)

First component to be measured

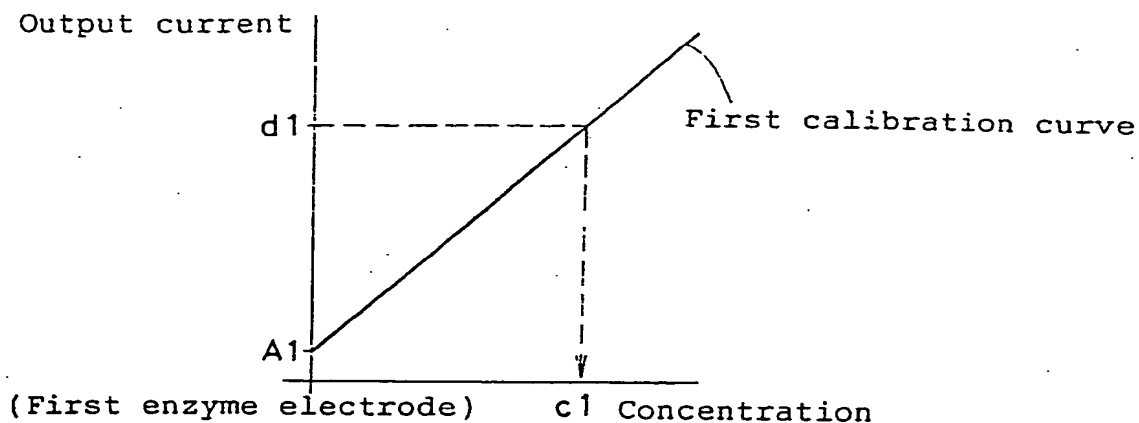


Fig.1 (2) Prior Art

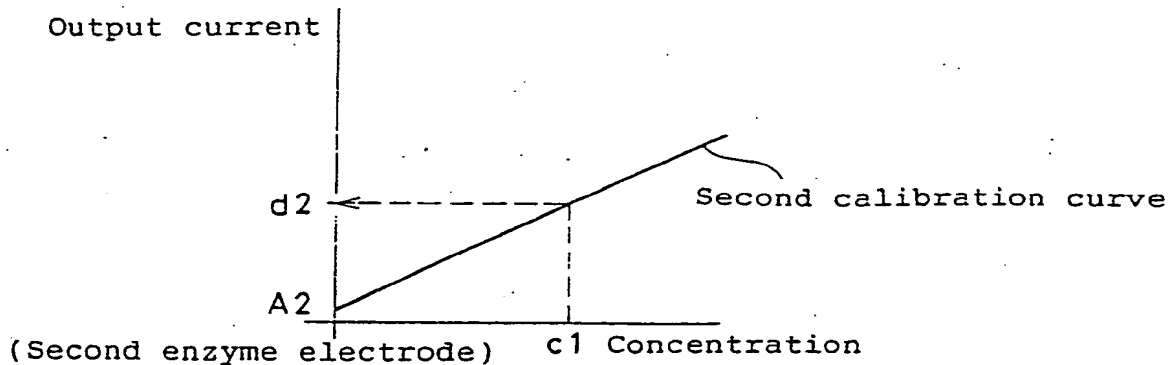
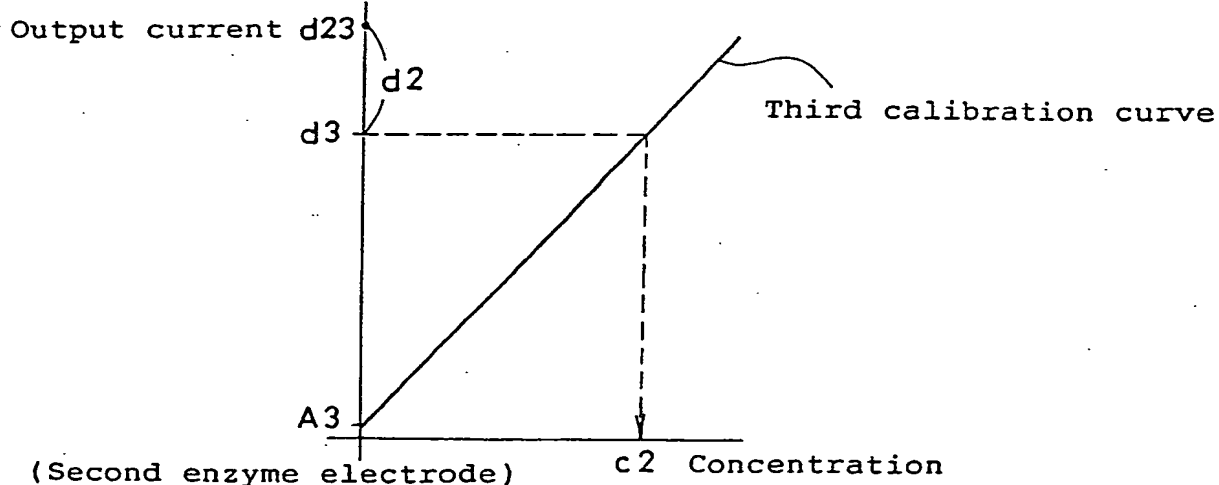


Fig.1 (3) Prior Art

Second component to be measured



*Broken line indicates the determination means.

Fig. 2

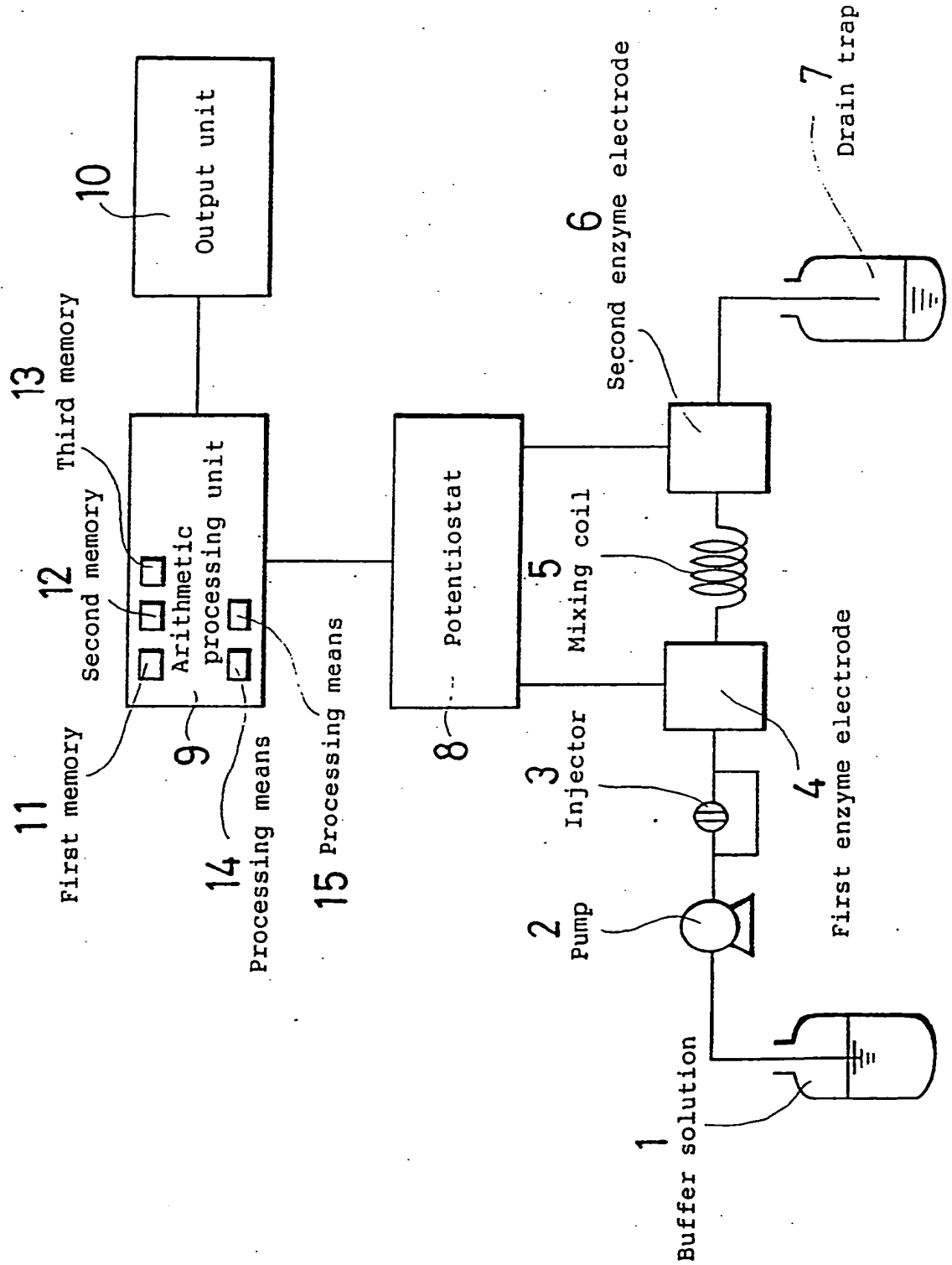
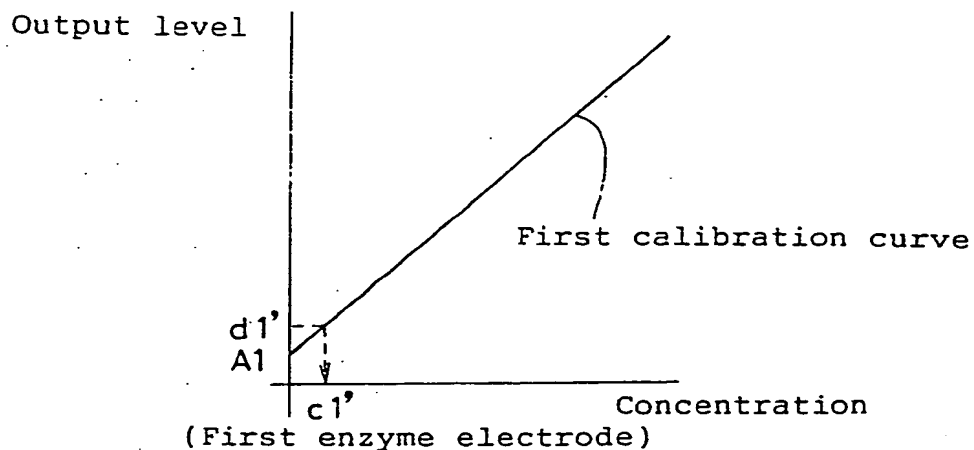


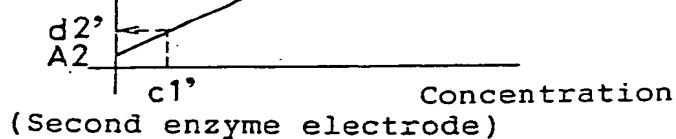
Fig. 3 (1) (Calibration means 2)

First substance to be measured

**Fig. 3 (2)**

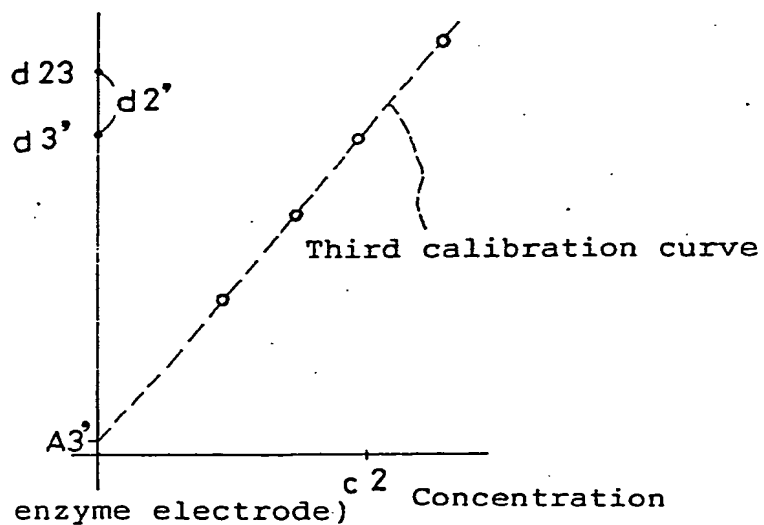
Output level

Second calibration curve

**Fig. 3 (3)**

Second substance to be measured

Output level



*Broken line indicates the determination means.

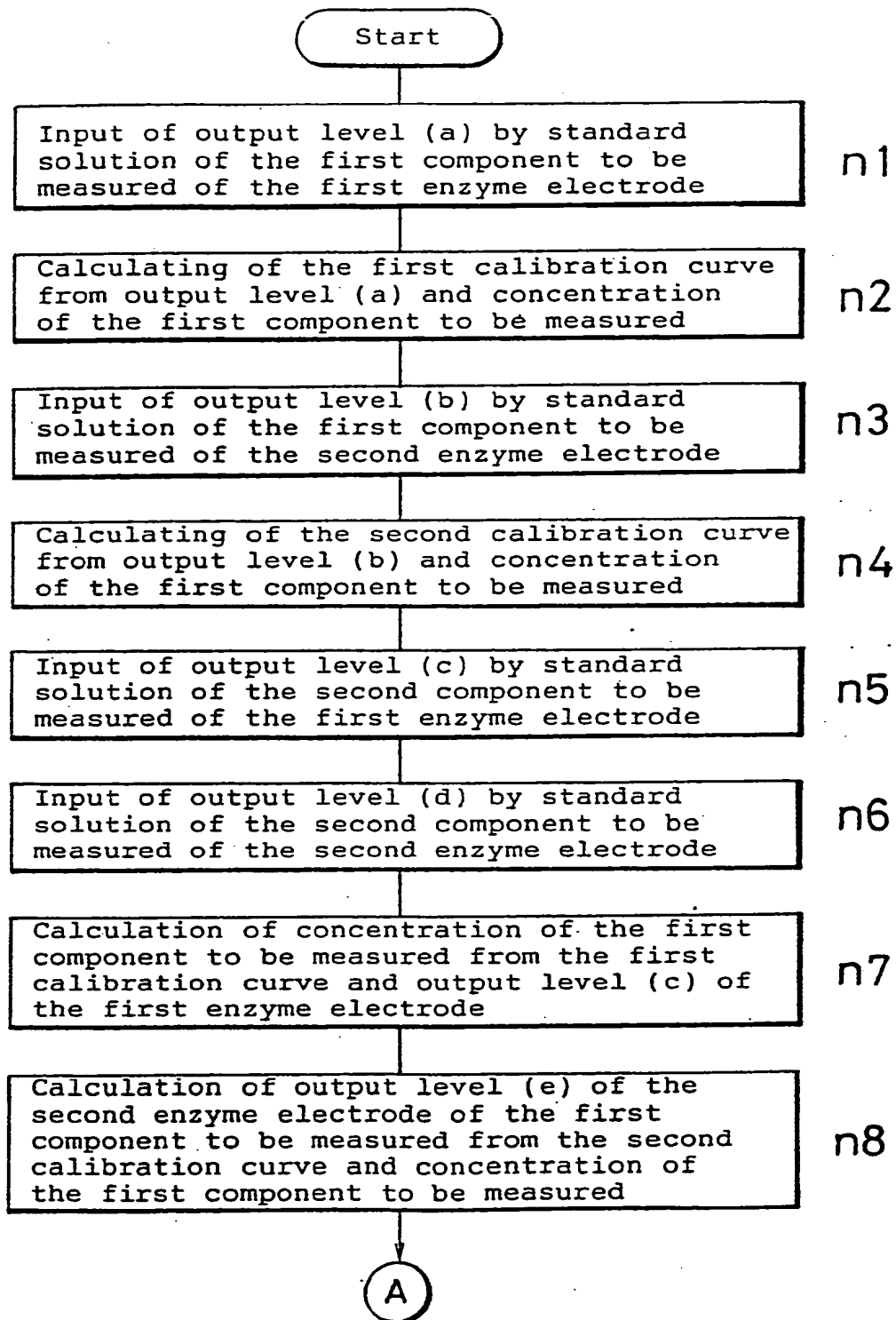
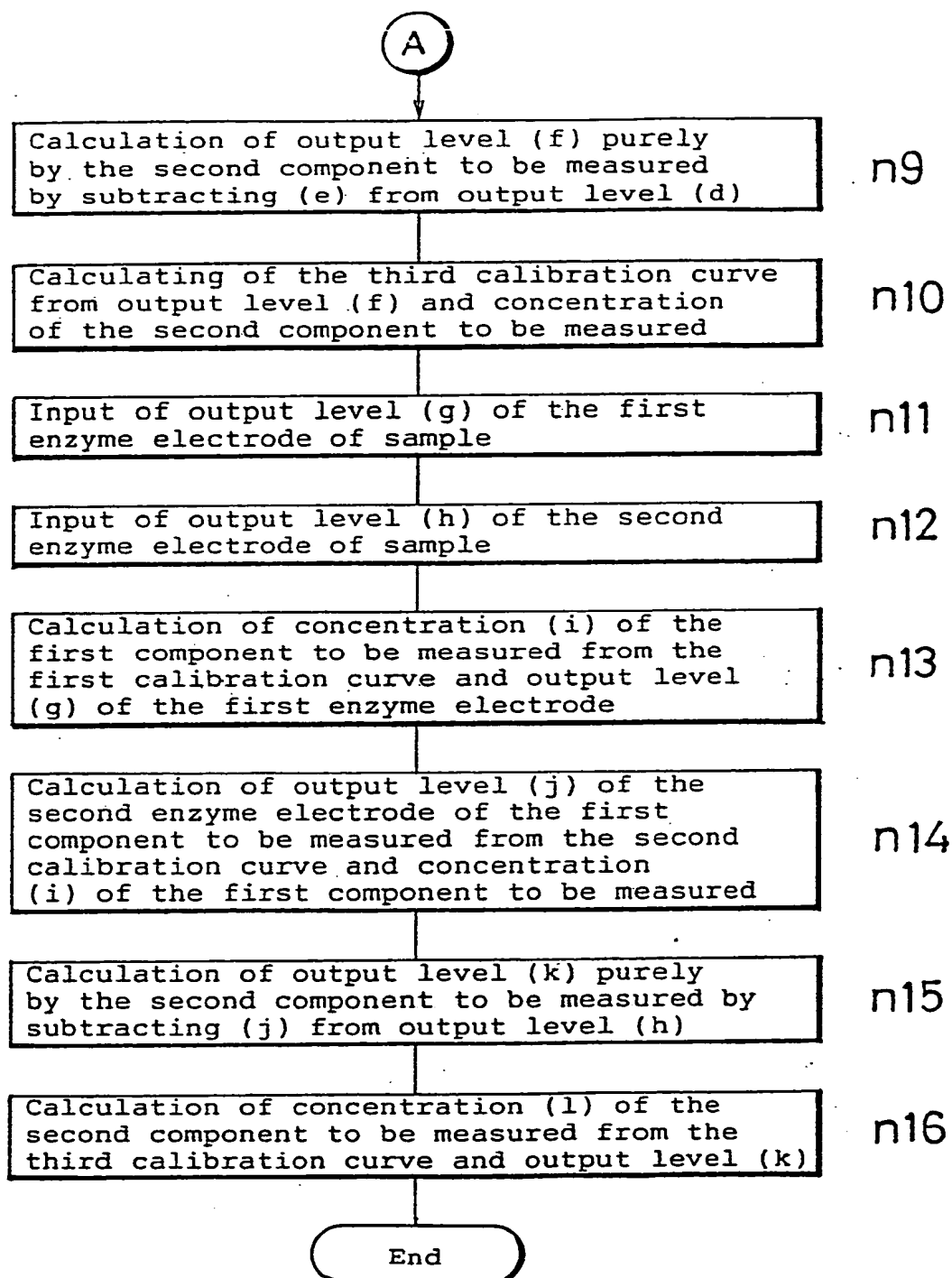
Fig.4 (1)

Fig. 4 (2)



n1 to n10 : Calibration means 2

n11 to n16 : Determination means 2



European Patent
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EUROPEAN SEARCH REPORT

Application Number

EP 89 12 2036

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	BIOTECHNOLOGY & BIOENGINEERING, vol. 25, 1983, pages 845-855, John Wiley & Sons, Inc., New York, US; J.P. KERNEVEZ et al.: "Determination of substrate concentrations by a computerized enzyme electrode" * Summary; page 851, line 8 - page 853, line 9; figure 7 * -----	1-4	C 12 M 1/40 G 01 N 27/28
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 12 M C 12 Q G 01 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 07-03-1990	Examiner EPAILLARD P.J.H.M.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	